

Vascular reconstitution in the tumor for more effective tumor immunotherapy

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Abstract

It has been widely accepted that the regulation of the tumor microenvironment is an important strategy in cancer treatment. Particularly, control of the tumor vasculature has been suggested to be critical for antitumor immunotherapy. Effectiveness of cancer immunotherapy depends on the quality and quantity of immune cells infiltrating into tumor tissues, which may be affected by the status of the tumor vasculature. Under physiological conditions, immune cells migrate from the intravascular lumen into the parenchyma especially by passing through the vascular wall of venules. Extravasation of immune cells is induced from venules where endothelial cells (ECs) are fully covered with pericytes from the basal side. Interaction of pericytes with ECs contributes to immune cell extravasation by several steps, ie, adhesion of immune cells to intraluminal ECs, transmigration, and chemotaxis of immune cells. Blood vessels are structurally immature and non-functional in tumors, and therefore, induction of maturation in the tumor vasculature is a promising strategy for effective cancer therapies and is relevant not only for immune cell migration but also drug delivery.

KEYWORDS

angiogenesis, blood vessel, endothelial cell, immunity, pericyte

1 | INTRODUCTION

Blood vessels play fundamental roles in homeostasis by supplying oxygen, nutrients, and immune cells to tissues on the one hand, and by removing carbon dioxide, waste products of metabolism, and excessive fluid on the other hand. Moreover, molecules secreted by endothelial cells (ECs) also have critical roles in maintaining organ integrity. Although coagulation factor VIII secretion from ECs in liver exerts its effects systemically, Wnt2 and bFGF from liver sinusoidal ECs are secreted as angiocrine factors to maintain hepatocyte survival and to regenerate the liver locally.¹ In cancer, secreted factors from tumor ECs support the growth of cancer cells and induce their stemness.² Therefore, aberrant overexpression of growth factors

from ECs has been suggested to be one of the hallmarks of tumor malignancy.

As mentioned above, blood vessels have pivotal roles for delivering immune cells to damaged or infected tissues. ECs of the venules determine which immune cells (ie, neutrophils, T cells, or monocytes) it is appropriate to deliver to tissues in specific circumstances by regulating the respective adhesion molecules for immune cell entry and maintenance of tissue integrity. Because ECs and immune cells have the same precursors, hemogenic angioblasts (previously known as hemangioblasts) from the mesoderm, these cells control vascular formation and immunity by mutually interacting. In cancer, vascular formation is affected by hematopoietic cells, especially by regulating matrix remodeling, growth of ECs,

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and other actions.^{3,4} Among the many aspects of vascular maturation, venogenesis (vein formation) is especially important in the consideration of tumor infiltrating lymphocytes mediating effective immune therapies.

In cancer, the effectiveness of chemotherapy depends on drug delivery into the tumor parenchyma. It is well established that drug delivery is reduced by increased interstitial hypertension.⁵ Vascular hyperpermeability in the tumor microenvironment is beneficial for macromolecule delivery of agents such as liposomes, but is detrimental for delivery of small molecule drugs and antibodies. Recently, improving the tumor microenvironment has been suggested to be critical for regulating vascular maturation to facilitate drug delivery and enhance tumor immune therapy.^{6,7}

Drugs are delivered from capillaries and immune cells infiltrate from venulae in normal organs. A common feature of capillaries and venulae is pericyte coverage of ECs in both types of blood vessel. In larger blood vessels such as arteries and veins, ECs are covered with smooth muscle cells. Direct cell-cell interaction between ECs and pericytes must be important for their specific vascular function in capillaries and venulae. Why is the maturation of blood vessels suppressed in the tumor microenvironment? How can we induce maturation of blood vessels in the tumor? In this review, we discuss these questions, with illustrations from our recent achievements in dissecting the molecular mechanisms of angiogenesis.

2 | CONSTRUCTION OF A HIGHLY HIERARCHICAL BLOOD VESSEL ARCHITECTURE

The vascular plexus, formed by vasculogenesis in an avascular area during the development of the embryo from the fertilized egg, changes its structure to a highly hierarchical blood vessel architecture ranging from small capillaries to larger arteries and veins through the process of vascular remodeling. In this process, when the formation of new blood vessels is required in the avascular field, branching of new blood vessels is induced from the existing vessel by what is known as sprouting angiogenesis. In both vasculogenesis and angiogenesis, the process of vascular maturation is induced by cell-cell interactions between vascular ECs and the mural cells surrounding them.⁸ A common feature of blood vessels is that ECs form a vascular tube in an intraluminal vascular cavity and mural cells adhere to ECs from the basal side (Figure 1). In capillaries and venulae, pericytes adhere to ECs and in larger blood vessels such as arteries and veins, it is smooth muscle cells that adhere to ECs. Pericytes and smooth muscle cells are collectively designated mural cells. After birth, the formation of new blood vessels in various different pathologies is induced by the process of angiogenesis, ie, sprouting and intussusception from existing blood vessels. Classically, it has been hypothesized that tumor cells co-opt pre-existing blood vessels to absorb nutrients to support their growth, but since around

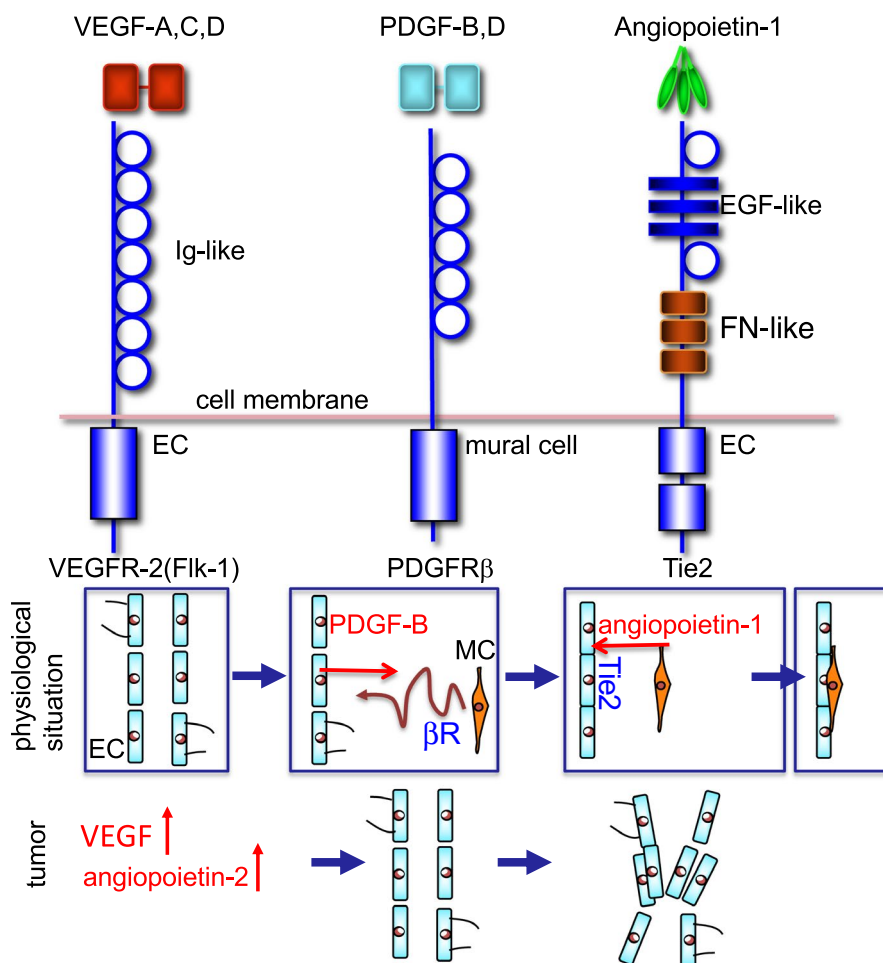


FIGURE 1 Comparison of vascular formation in the normal and the tumor microenvironment. Maturation by PDGF-B/PDGFR β and angiopoietin-1/Tie2 of blood vessels induced by VEGFs/VEGFR2. PDGF-BB recruits mural cells (MCs) expressing PDGFR β near to endothelial cells (ECs). Under physiological conditions, MCs secrete angiopoietin-1 to induce activation of Tie2 that facilitates EC-EC adhesion, finally resulting in the covering of ECs by MCs. In tumors, MC coverage of ECs is suppressed by overexpression of VEGF and angiopoietin-2, thereby maintaining immature vessel characteristics

1970, attention has been focused more on the induction of blood vessel formation by sprouting angiogenesis and the elucidation of the mechanisms involved in this type angiogenesis has proceeded apace.⁹

In the process of angiogenesis, blood vessel formation involves mainly 3 types of vascular ECs.¹⁰ Vascular endothelial growth factor (VEGF) is secreted from tumor tissue due to ischemia induced by cancer cell growth; it acts on pre-existing blood vessels and induces the generation of tip cells expressing filopodia on the cell surface. In addition to tip cells, ECs termed "breach" cells have been identified by single cell RNA sequencing of ECs present in the tumor microenvironment; they are suggested to be involved in matrix remodeling.¹¹ Tip cells play a role in guiding vascular branches to areas where new blood vessels are required. The cells that are in close contact with the tip cells, just behind them, are called stalk cells, which have a marked ability to produce vascular ECs and are involved in the elongation of new blood vessels. We have explored the origin of these highly proliferative cells and determined that they are derived from vascular endothelial stem cell-like cells present in the vascular ECs of existing blood vessels.¹²⁻¹⁴

For physiological angiogenesis, amelioration of ischemia or inflammation results in maturation or regression of new blood vessels. For transient tissue formation, unnecessary blood vessels regress due to apoptosis of ECs. Conversely, to maintain new blood vessels in the organ even after ischemia is resolved, the vascular structure undergoes a maturation process characterized by the appearance of vascular ECs termed phalanx cells.¹⁵ VEGF activates VEGF receptor 2 (VEGFR2) on ECs, which causes intracellular signal transduction by activating the Src tyrosine kinase and results in phosphorylation and internalization of VE-cadherin.¹⁶ In this manner, VEGF causes vascular leakage by inducing vascular hyperpermeability. Phalanx cells increase the expression of VE-cadherin to induce blood vessels with less permeability and thereby generate mature blood vessels in which ECs are fully covered by mural cells.

For maturation of the blood vessels, 2 important molecules are platelet derived growth factor (PDGF)-BB and angiopoietin-1⁸ (Figure 1). PDGF-BB released from ECs promotes recruitment of mural cells expressing PDGFR β near to the ECs, and angiopoietin-1 from mural cells activates Tie2 on ECs. Together, this results in EC:EC adhesion by stabilizing VE-cadherin on the cell membrane by mDia.¹⁶ In addition to these molecules, different cytokines and lipid mediators also function to induce stabilization of the vascular structure. Eventually, the mural cells adhere to ECs and the extracellular

matrix wraps around the blood vessels, terminating the maturation mechanism of the completed vascular structure. Matrix production has been shown to be induced by TGF β , but it is not yet clear how direct cell-cell adhesion between mural cells and ECs is induced.

3 | FEATURES OF TUMOR VASCULAR STRUCTURE AND FUNCTION

Unlike normal tissue blood vessels, those in tumors exhibit various different structural abnormalities such as meandering and dilation, sudden occlusion, intravascular vascular proliferation, and excessive vascular branching in multiple directions. Despite such abnormal structural features, it is widely accepted that blood vessels formed in tumors do supply cancer cells with plentiful oxygen and nutrients, and are closely related to the growth of the cancer tissue. Conversely, it has been reported that intratumoral blood vessels manifest insufficient connectivity between the vascular ECs forming the lumen and, therefore, vascular permeability is enhanced, allowing fluid to enter the tumor parenchyma. This results in edema and causes a higher interstitial fluid pressure compared with blood vessels in the normal organ.¹⁷ The transient hypervascular permeability permits delivery of molecules into the parenchyma, and it was thought that this made it likely that large amounts of oxygen become available to the cancer tissue. However, this interpretation is not correct.

For the transport of oxygen and drugs from blood vessels to tissues, tight adhesions between vascular ECs would need to be induced for the maintenance of higher intravascular pressure (Figure 2). Based on the lower interstitial fluid pressure compared with intravascular higher pressure, oxygen leaking from the gap between ECs can penetrate into the parenchyma by diffusion along the pressure gradient. Delivery of small molecule drugs (including antibodies) also proceeds in the same manner, ie by diffusion. It is possible for high-molecular-weight drugs (liposomes, etc.) to pass through and enter the tumor through the large gaps between vascular ECs, as observed in tumor tissue, but the opposite is true for low-molecular-weight compounds. In summary, in the tumor microenvironment, hypoxia is induced and drug delivery is impaired. Hypoxia increases tumor cell malignancy by promoting chromosomal instability and mutation and, therefore, sensitivity to anticancer drugs is reduced in cancer cells in such a sub-optimal microenvironment.

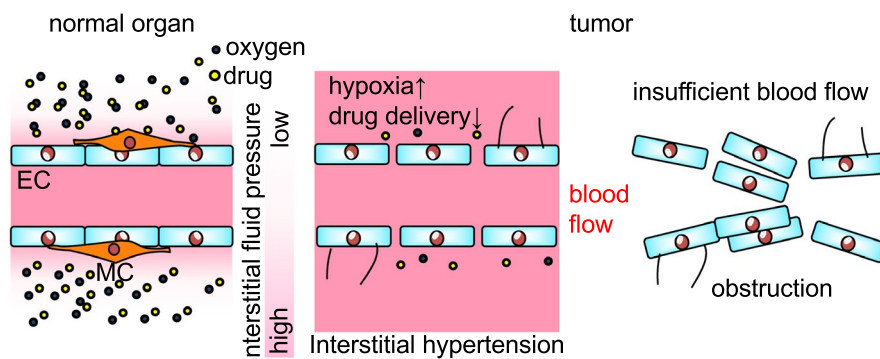


FIGURE 2 Impaired perfusion and drug delivery in the tumor microenvironment. Oxygen penetration and drug delivery from blood vessels are severely impaired by the interstitial hypertension prevalent in the tumor microenvironment. Insufficient perfusion by the irregular and abnormal structure of blood vessels in tumors enhances this impairment of drug delivery and oxygenation

How are structural abnormalities of blood vessels in tumors induced? Many causes have been pointed out, but we suspect that insufficient coverage of vascular ECs by mural cells is one major reason why blood vessels exhibit abnormalities (Figure 2). One of the major mechanisms of pericyte loss is thought to be that the maturation process of blood vessels is suppressed, but that VEGF expression is maintained. Because the growth of cancer cells and stromal cells is sustained in the tumor, hypoxia is continuously induced and VEGF expression is maintained by stabilization of hypoxia-inducible factor 1 α (HIF1 α). It has been suggested that tumor stromal cells mainly produce VEGF, however activation of growth factor receptors such as epidermal growth factor receptor (EGFR) also positively regulates VEGF expression independent of HIF1 α .¹⁸

In addition to continuous overexpression of VEGF, high expression of angiopoietin-2 (Ang2) is also considered to be a factor contributing to suppression of mural cell adhesion (Figure 1). Ang2, like Ang1, binds to Tie2, but has a weak ability to induce phosphorylation of Tie2 and is considered to be an antagonist of Ang1 (Ang2 may be an agonist of Tie2 in a context-dependent manner). Ang2 is also a molecule, the expression of which is enhanced during hypoxic responses. In other words, the increase in VEGF and Ang2 is thought to prevent the maturation of blood vessels in the tumor. Indeed, it has been reported that simultaneous inhibition of VEGF and Ang2 further promotes the maturation of tumor blood vessels compared with the suppression of VEGF-based signals alone.¹⁹ The induction of vascular maturation and characteristics of the cancer microenvironment are further outlined below.

4 | VASCULAR NORMALIZATION OR VASCULAR PROMOTION IN THE TUMOR MICROENVIRONMENT

Maturation of the tumor vasculature should decrease the interstitial hypertension by drainage of fluid from the tumor parenchyma and,

therefore, improve drug delivery into the tumor tissue.²⁰ The concept of vascular function improving after anti-angiogenic therapy was suggested by evidence that suppression of VEGF-based signals and the combined use of anticancer drugs have a more pronounced antitumor effect than anticancer drugs alone in clinical trials.²¹ Although this concept seems to be correct, VEGF signal blockade also causes the destruction of newly developing blood vessels and, therefore, upon treatment with angiogenesis inhibitors it induces a mixed state of suppression and normalization of tumor vasculature in the tumor microenvironment.²²

To test the concept of vascular normalization, it is theoretically correct to observe the effects on the tumor environment of molecules that induce physiological vascular maturation. Therefore, we first focused on the Tie2 receptor and showed that its activation in vascular ECs does indeed induce vascular maturation by promoting tight EC-EC adhesion, as described above. However, Tie2 activation also induces vascular sprouting in a context-dependent manner in which ERK activation is more strongly induced than in the PI3/Akt pathway in ECs.²³ Therefore, we analyzed the former signal that induces maturation and found that a physiologically active peptide called apelin was secreted from vascular ECs on activation of Tie2.²⁴ Apelin is a ligand for APJ, a 7-transmembrane G protein-coupled receptor expressed on ECs when angiogenesis is ongoing. We found that when APJ was activated, VEGF-induced internalization of VE-cadherin into the cytoplasm was suppressed.²⁵ Moreover, by analyzing apelin knockout (KO) or transgenic mice we found that apelin induced cell-cell aggregation of ECs, resulting in the formation of mature capillaries with large diameters.²⁴ Furthermore, we discovered a crucial role for apelin/APJ in promoting parallel juxtapositional alignment between arteries and veins,²⁶ one feature of well organized vascular structures. Therefore, apelin is a maturation factor for blood vessels. We found that apelin overexpression in tumors enhanced blood flow by inducing healthy blood vessels, resulting in a reduction in hypoxia and improvement of drug delivery²⁷ (Figure 3).

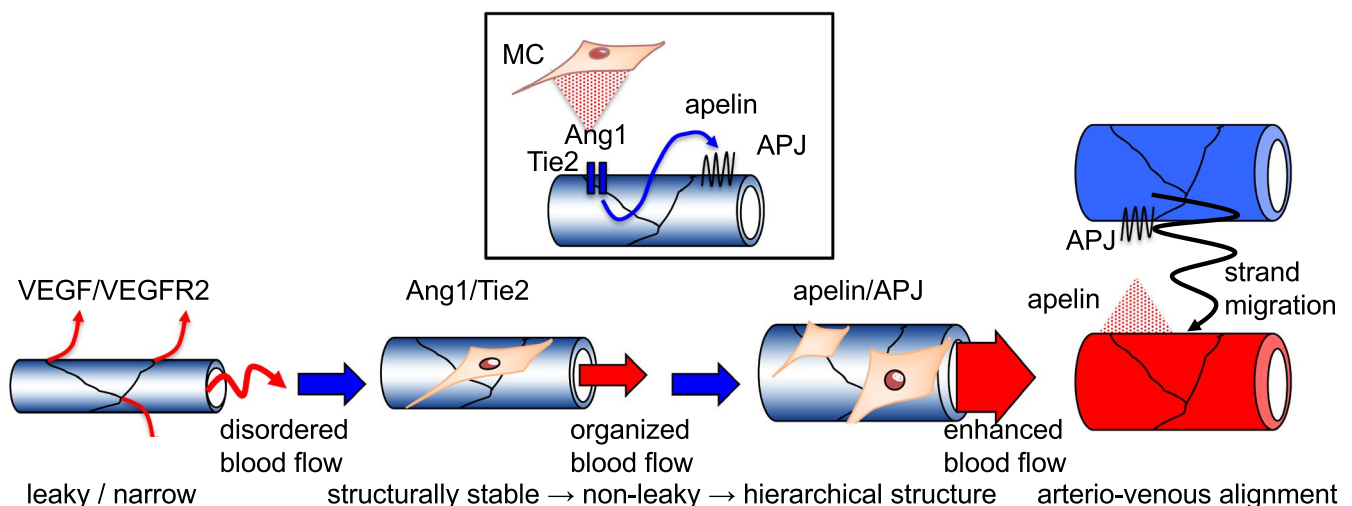


FIGURE 3 Maturation of blood vessels by the Ang1/Tie2 and apelin/APJ axes. Ang1/Tie2 induces structuredly stable blood vessels and promotes production of apelin from ECs. Upon stimulation of APJ by apelin in ECs, the diameter of capillaries is enlarged. Apelin produced by ECs of arteries promotes vessel strand migration of venous ECs expressing APJ. Taken together, highly hierarchical structures of blood vessels ranging from small capillaries to larger venules and arterioles are induced together with arterio-venous alignment

Additionally, we identified the lipid mediator lysophosphatidic acid (LPA) by screening for molecules that enhanced adhesion between vascular ECs and contributed to the suppression of vascular permeability in tumors.⁶ When LPA binds LPA receptor 4 (LPA4), activation of G12/13 transmits a signal to Rho/ROCK to assemble actin directly under the vascular endothelial cell membrane; VE-cadherin is then recruited to that location, resulting in the formation of a zipper-like structure for EC adhesion (linear adhesion band). Activation of LPA4 led to the formation of a healthy vascular network within the tumor by connecting non-functional blood vessels that had previously been dead ends with suppressed blood flow. After improvement of blood flow by LPA, capillaries restore drainage function, induce a decrease in interstitial hypertension in the tumor, reduce hypoxia, and mediate a marked antitumor effect in combination with anticancer drug treatment (Figure 4).

To summarize this part of the review, it is now established that the tumor microenvironment is certainly improved by inducing vascular maturation and promotion of normal blood vessel formation, very different from the aim of suppressing VEGF-based signals. In consideration of these data, vascularization-promoting therapies will be developed for reconstructing normal blood vessels in tumors as a new category of tumor treatment in combination with anticancer drugs in future. Potentially supplementary to this, combinations with other drugs that have been reported to induce tumor vasculature maturation, such as the anti-malarial drug chloroquine or the RGD peptide that binds to integrin, may conceivably further enhance these effects.^{28,29}

5 | TUMOR VASCULAR REGULATION AND TUMOR IMMUNITY

Reciprocally, it has also been found that attenuation of tumor immunity is associated with abnormalities and non-functionality of tumor

blood vessels. In the context of tumor immunity, important relationships with changes in macrophage polarity have been established. Macrophages of the so-called M2-like type often predominate in the tumor microenvironment, inducing regulatory T cell infiltration and suppressing tumor immunity.³⁰ We found that Gal-3, a galactose-binding lectin, is involved in the polarization of these macrophages in tumors. Briefly, when tumor cells expressing Gal-3 were inoculated into Gal-3 KO mice, Gal-3 formed a concentration gradient in tumors into which predominantly M2-like macrophages migrated, resulting in disorganized vascular proliferation and tumor growth.³¹ This factor is one piece of evidence for enhanced tumor malignancy instigated by M2-like macrophages.

It has been reported that VEGF also impacts on macrophage polarity. Therefore, when VEGF/VEGFR signaling is prevented in the tumor, conversion of macrophages from M2-like to M1-like type is induced and antitumor immune effects are enhanced.³⁰ It has also been shown that VEGF signals are involved not only in macrophage polarity changes, but also in influencing CD8-positive lymphocytes and regulatory T cells. For example, overexpression of VEGF and prostaglandin E₂ in the tumor microenvironment induces the expression of FasL by the normally FasL-negative vascular ECs of the tumor blood vessels. This results in abrogation of CD8-positive cytotoxic T cell infiltration into the tumor by inducing apoptosis of these cells by Fas-FasL interactions³² (Figure 5). Conversely, regulatory T cells with high expression of c-Flip, which suppresses caspase expression, pass through FasL-expressing vascular ECs without being killed, and the tumor microenvironment becomes immune tolerant. This phenomenon was designated a "selective immune barrier" suppressing tumor immunity. Therefore, it has been reported that suppression of VEGF and prostaglandin E₂ restores tumor immunity by inhibiting FasL expression by tumor ECs.

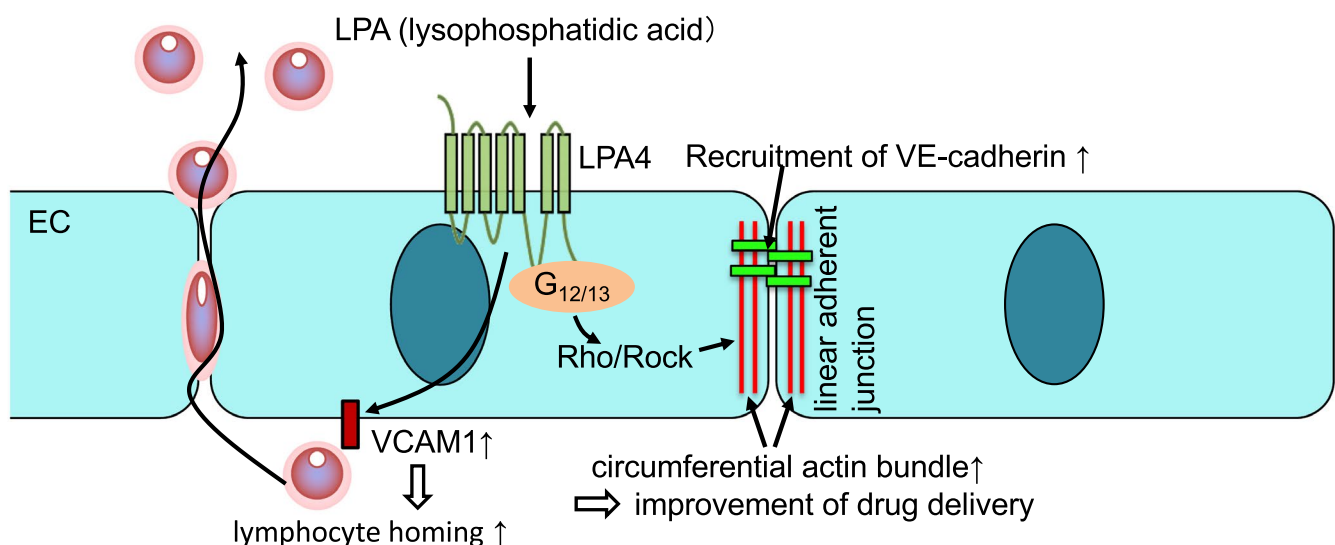
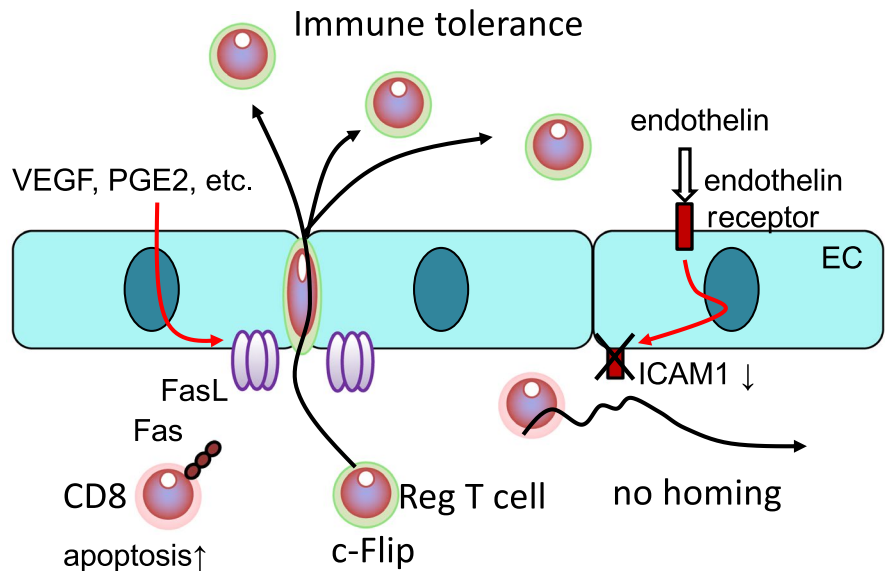


FIGURE 4 LPA4 activation of ECs induces fine capillary formation. Activation of LPA4 expressed on ECs by LPA results in improvement of drug delivery from blood vessels by the formation of linear adherent junctions between ECs through G_{12/13} and Rho/Rock activation. Leukocyte infiltration into the tumor microenvironment is facilitated through VCAM-1 expression on ECs, resulting in effective tumor immune therapy

FIGURE 5 Selective immune barrier formation by continuous expression of inflammation-relevant proangiogenic molecules. Overexpression of cytokines and inflammatory factors, ie, VEGF and PGE2, induces FasL expression by ECs that causes apoptosis of CD8-positive cytotoxic T cells upon their transmigration and contact with tumor ECs. Regulatory T cells expressing c-Flip gain free access to the tumor microenvironment. Endothelin attenuates expression of ICAM1 in ECs, resulting in the abrogation of lymphocyte homing to tumor ECs



Inhibition of FasL expression by ECs following VEGF/VEGFR2 signal perturbation is one means of inducing functional normalization of the tumor vasculature. In addition, we have also shown that tumor immunity is activated on regulation of tumor vascularity by apelin and LPA. For example, for apelin, when APJ activation is induced from the early stage of tumor development by overexpression of apelin in the tumor, hierarchical vascular structures with large, medium and small blood vessels are induced as described above, and intratumoral blood flow is normalized. When tumors with high amounts of apelin were generated and activated dendritic cells (DCs) were infused into tumor-bearing mice, abundant natural killer (NK) cells were found to infiltrate the tumor and its growth was effectively suppressed.²⁷ We have established that apelin/APJ is involved in the maturation of those blood vessels that are in close proximity to arteries and veins and, therefore, venous formation by apelin may be a key regulator of immune cell infiltration.

Moreover, we have found that LPA4 activation by LPA induces mature vascular network formation in the tumor, as described above. In addition to its effects on blood flow in the tumor, we found that activation of LPA4 in vascular ECs induces the expression of VCAM1. This is required for lymphocyte adhesion, which promotes infiltration of CD8-positive cells into the tumor.⁷ When an LPA4 agonist and an immune checkpoint inhibitor were simultaneously administered, tumor growth was markedly suppressed. This result is consistent with analogous findings regarding immune cell adhesion factors. For example, bFGF secreted from tumor tissue induces the expression of endothelin by cancer cells and activates endothelin receptors on vascular ECs and, therefore, suppresses the expression of ICAM-1. This finally results in attenuation of leukocyte infiltration³³ (Figure 5). The arterioles lead to the capillaries that then converge on venules. The pressure in the capillaries on the arterial side is 25 mmHg and 15 mmHg on the venous side. Blood flow slows down in the venules and therefore leukocytes have more opportunity to attach to vascular ECs there. When inflammation is induced by foreign

agents, selectin family protein expression on venule ECs is upregulated, promoting leukocyte rolling. These leukocytes strongly adhere to ECs expressing VCAM-1 and ICAM-1, and diapedesis into the parenchyma follows. The control of which leukocytes should infiltrate the tissues is by means of chemokine expression at the foci. Most leukocytes, such as T cells, neutrophils, and monocytes, utilize this system for extravasation, however the manner of invasion is different for different cell types. Monocytes that finally differentiate into macrophages change their shape/morphology for diapedesis and infiltration into the parenchyma without remodeling their extracellular matrix, which in the steady state is that based on their original role of patrolling alongside blood vessels. Conversely, neutrophils and T cells remodel their vascular basement membrane to allow a route for their penetration and migration.³⁴ However, these mechanisms have all been established by observations on normal organs. Differences in the extravasation among leukocyte types have not been well clarified. Further analysis is required to understand the mechanisms of leukocyte infiltration into the tumor microenvironment.

Control of tumor immunity by such vascular regulation is currently a very hot topic. The formation of immune hotspots and of high endothelial venules (HEVs) in tumors is now overviewed.

6 | FORMATION OF HEVS IN THE TUMOR MICROENVIRONMENT

Continuous immune cell entry into and exit from the lymph nodes is a crucial foreign body monitoring mechanism. Foreign antigens/foreign substances are carried into the lymph nodes by lymphatic vessel drainage. Antigen-presenting cells (APCs), such as DCs activated by foreign antigens in tissues, also migrate to the regional lymph nodes, which is where immune cell activation occurs in response to these antigens on the APCs. Importation of lymphocytes through HEVs into the lymph nodes is induced by several chemokines such as

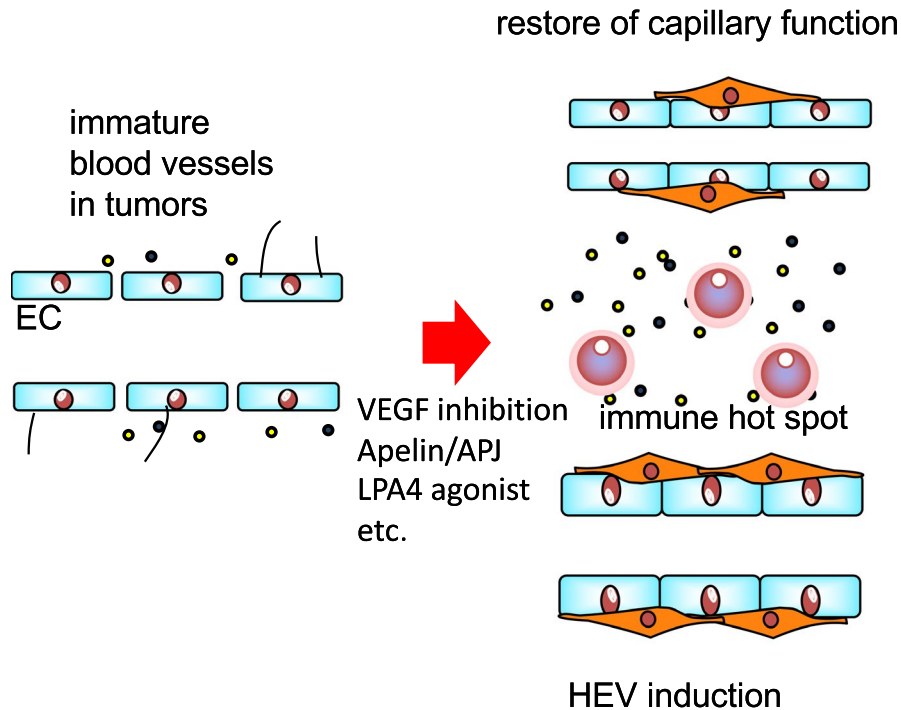


FIGURE 6 Concept of immune hot-spot formation by blood vessel maturation factors. The formation of mature capillaries and venules including HEV is critical for immune hot-spot formation in the tumor microenvironment. Pericyte coverage is one of the key events for maturation of blood vessels within the tumor, which might be induced by orchestrating the application of vascular maturation factors. Drug delivery and hypoxia need to be improved to enhance the efficacy of immune therapies

CC-chemokine ligand 21 (CCL21), CCL19 and CXC-chemokine ligand 13 (CXCL13).³⁵

The blood vessels in the lymph nodes, which as noted above allow lymphocyte invasion, are termed HEV, and are morphologically and functionally slightly different from the venules of normal tissues.³⁴ The mural cells that cover these vascular ECs are not pericytes, but reticular fibroblasts densely surrounding them. Unlike other venules, the cytoplasm of ECs from HEVs is characteristically thicker than that of venule ECs. HEV ECs express an oligosaccharide with sulfate groups and sialic acid hexasulfated sialyl-Lewis X (sLeX), ligands for L-selectin that are involved in lymphocyte rolling and homing to ECs. This oligosaccharide modifies core proteins expressed on ECs such as GlyCAM-1, CD34, endomucin, and podocalyxin. HEVs therefore promote infiltration of lymphocytes into lymph nodes by chemokines such as CCL21, CCL19, CXCL12, and CXCL13 (some of which are secreted by vascular ECs themselves) and integrin ligands such as MadCAM-1 and ICAM-1.

HEVs have gained attention in the tumor biology field because it has become clear that they are closely associated with lymphocyte infiltration into tumors. In many cancer types, it has been found that they form tertiary lymphoid structures and are involved in the formation of immune "hot spots."³⁶⁻³⁸ The immune checkpoint mechanism is a system involved in autoimmunity by suppressing the activation of CD4 and CD8 lymphocytes expressing PD-1, and other receptors, but it is also a mechanism that maintains tolerance and blocks antitumor immunity.³⁹ While marked antitumor effects of immune checkpoint inhibitors have been reported, many patients still do not benefit from them. One reason for this is that lymphocytes do not invade the tumor and therefore cannot attack the cancer cells. Therefore, induction of the formation of immune hotspots is considered to be a method for enhancing the effect of immune checkpoint inhibitors (Figure 6).

So far, it has been reported that HEV are present not only in normal tissues such as lymph nodes, but also in lesions of rheumatoid arthritis, inflammatory bowel disease, bronchial asthma, autoimmune thyroiditis, *Helicobacter* gastric inflammation, and many other chronic inflammatory diseases.³⁵ Chronic inflammation is caused by prolonging inflammation effected by lymphocyte infiltration into tissues and, under these circumstances, it is considered that suppressing the formation of HEV will help to resolve the pathological condition.

However, it has recently been reported that HEV-like blood vessels are also present in cancer tissues such as breast cancer, colon cancer, lung cancer, ovarian cancer and melanoma, and that CD8-positive lymphocytes are found in the vicinity of HEVs. Clinical evidence suggests that the abundance of HEV-like blood vessels is positively correlated with a better prognosis.⁴⁰ Therefore, one of the reasons why the effects of tumor immunotherapy such as cancer vaccines have been limited in the past is the suppression of HEV-like angiogenesis in tumors. The induction of HEV-like blood vessels in tumors has been recognized as important for further enhancing the effects of immune checkpoint inhibitors. For that purpose, it is vital to characterize the interactions between immune cells and vascular ECs for the generation of HEV.

In the context of interactions between vascular ECs and hematopoietic cells (including immune cells in a wider sense), we have determined that vascular growth factors such as Ang1 secreted by hematopoietic stem cells are involved in the construction of the vascular network.^{41,42} Furthermore, in relation to VEGF, we found that Neuropilin-1 (NRP1), which is one of the VEGF receptors, is expressed on T and B lymphocytes. When VEGF binds to NRP1 on lymphocytes and this VEGF and NRP1 complex stimulates ECs, stronger phosphorylation is induced compared with VEGF alone.⁴³ These

mechanisms suggest that the blood cells surrounding the vascular ECs function as accessory cells during the process of angiogenesis.

Similarly, immune cells may be involved in the formation of HEVs in tumors. It has been suggested that DCs present in the tumor play a role in inducing HEV characteristics in ECs through their expression of lymphotoxin (LT) that ligates the LT receptor β expressed on ECs.⁴⁴ Moreover, it has been reported that type 1 helper T cells (Th1) produce interferon (IFN) γ and stimulate CXCL9, CXCL10, and CXCL11 chemokine secretion from the stromal cells residing beside the ECs. In this way, Th1 cells positively regulate lymphocyte infiltration into the tumor.^{44,45} Furthermore, it has been suggested that IFN γ from Th1 cells also inhibits VEGF expression in stromal cells to suppress abnormal vascular formation. In terms of VEGF, DC maturation from immature DCs is suppressed by VEGF. Mature DCs express LT and, therefore, positive feedback regulation of mature DCs and Th1 cells seems to induce HEV in the tumor. However, how pericytes and/or reticular fibroblasts are recruited during HEV formation has not been clearly established. The precise molecular mechanism of how HEVs are formed in tumor tissues, including dissecting how mural cells adhere to ECs, must be elucidated to understand how HEVs are induced in tumors.

7 | CONCLUSIONS

Basic research has shown that vascular normalization by VEGF inhibition and immune checkpoint inhibition is effective in promoting HEV formation.⁴⁶ Recently, therapeutic protocols using angiogenesis inhibitors in combination with immune checkpoint inhibitors have been developed and their effectiveness documented in several cancer types.⁴⁷ In this review, we describe the basic mechanisms of angiogenesis and the effects of tumor vascular regulation by vascular maturation factors. ECs, even in a normal organ, are genetically and phenotypically heterogeneous, but more so in tumors because of the abnormal microenvironmental conditions. We have also recently reported that vascular endothelial stem cells are present in pre-existing blood vessels, causing vigorous tumor angiogenesis.¹²⁻¹⁴ In addition, regarding heterogeneity, new types of ECs have been identified based on single cell RNA analysis of vascular ECs in tumors.¹¹ Because of the heterogeneity of blood vessels in tumors, it would be expected that tumor immunity must also be heterogeneous even within the microenvironment of the same tumor. A more complete understanding of the mechanisms of angiogenesis, including venogenesis and HEV formation, as well as immunity, will be furthered by clarifying the function of these different ECs.

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CONFLICT OF INTEREST

The author has no conflicts of interest to declare.

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